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Color and pattern variation of the Balkan whip snake, *Hierophis gemonensis* (Laurenti, 1768)

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Abstract: In the present paper color and pattern variations of *Hierophis gemonensis*, a snake with little morphological variation, are described. For the first time, partially melanistic individuals were observed and described for this species. The identity of one of these snakes was corroborated by genetic means. Taxonomical circumstances and environmental causes of this color and pattern variation are discussed.

Key words: Phenotypic plasticity, taxonomy, environment, mtDNA, Colubridae, Balkan Peninsula

Many reptile species display high genetic but low phenotypic variation, which is often reflected in a general mismatch between taxonomic and genetic differentiation. Current genetic research has resulted in taxonomic changes for many European species including their synonymization, elevation to higher levels, or description of newly recognized hidden phylogenetic clades (see Torstrom et al., 2014 for a review). Deep genetic divergences among several European reptiles were uncovered only recently due to little differences in their phenotypes (see Gvoždík et al., 2013; Ghielmi et al., 2016). This suggests, surprisingly, how often the evolution of genotype does not correspond to the evolution of phenotype. On the other hand, some species are phenotypically very variable but genetically more uniform (e.g., *Testudo graeca*: Fritz et al. 2007; Mikulíček et al. 2013; *Natrix natrix*: Kindler et al., 2013). However, different colorations in similar species can also reflect deep speciation processes (e.g., *Dasypeltis*: Trape et al., 2012). Therefore, taxonomic changes without genetic support should be based on comprehensive data covering the morphology, biogeography, and ecology of particular species, which has not always been complied previously.

The Balkan whip snake, *Hierophis gemonensis* (Laurenti, 1768), occurs in a coastal strip from southern Slovenia through Croatia, Bosnia and Herzegovina, Montenegro, Albania, and Macedonia to Greece (including Crete and various smaller islands of the Adriatic, Ionian, and Aegean seas; Sillero et al., 2014). It is the most frequently observed snake species in phrygana and degraded maquis vegetation and in cultivated land from lowlands (most common) up to 1400 m elevation. As Henle (1993) pointed out, this species shows little morphological variation with respect to body size, meristics, pattern, and coloration. To our best knowledge, no color aberrations (albinism, amelanism, erythrism, melanism, etc.) or variations were recorded for this species (Henle, 1993), except for a doubtful report by Schimmenti and Fabris (2000) who mentioned (without any details) a melanistic individual of *H. gemonensis* from Krk Island (Croatia). However, there is possible confusion with *H. carbonarius* because both species occur on the island of Krk. In any case, no unambiguous record of a melanistic *H. gemonensis* has been published yet. Recent phylogeographic investigations showed only a low level of DNA sequence divergences (mitochondrial DNA), which could be connected with phenotype differentiation, localized in the southwestern part of the Balkans and on Crete (Kyriazi et al., 2013; Mezzasalma et al., 2015).

During field work campaigns in the Balkans we recorded extraordinarily colored specimens of whip

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snakes that were tentatively identified in the field as *H. gemonensis*. The snakes were identified according to meristics and coloration of the body presented by Henle (1993) and Arnold and Ovenden (2002). In total, three records were collected by the authors where locality, coordinates, altitude, sex, age, basic morphological data, and photographs were taken, and ten further unpublished observations were provided by colleagues (see the Table and Acknowledgments). To find out the approximate frequency of this coloration in nature, we used observation data from Balcanica, an online mapping project of amphibians and reptiles in the Balkans (http://balcanica.info), and our own unpublished dataset. We sequenced the mitochondrial marker cytochrome *b* (cyt *b*) of one unusually colored specimen from Divjakë-Karavasta National Park, Albania (for the PCR protocol and further details see Nagy et al., 2004) and compared it with the dataset of Kyriazi et al. (2013). We also constructed a haplotype network using the 95% limit of parsimony (not shown in this study) as implemented in TCS 1.21 (Clement et al., 2000) to find out the haplotype affiliation of the specimen. This specimen was also morphologically examined and compared with published data. All captured snakes were released back to nature.

In total, 345 specimens of *H. gemonensis* have been recorded including data presented in the Table of this paper, our own unpublished field observations, and data of Balej and Jablonski (http://www.balcanica.cz). From this dataset, 13 (3.8%) specimens were unusually dark in color (partially melanistic) and they were recorded in Albania, Croatia, Greece, and Montenegro (Figure 1). None of the individuals were completely black (Figures 2A–2H and 3A–3H). All individuals described here showed markedly dark coloration with a pattern that was fading towards the back of their bodies (Figures 2A, 3B, 3D, and 3E). The pattern was composed of a line running dorsally along midbody and with two rows of small or bigger blotches occurring more laterally (Figure 2E). In the front portion of the body the blotches and line were fused and they formed a zig-zag pattern, which is similar to what we can find in the genus *Vipera* Laurenti, 1768 (Figures 2E and 3E). Between the dorsal and ventral parts of the body there is a sharp color contrast, sometimes presented by a black line (Figure 3B). The ventral part of the body was white with scattered gray blotches (Figures 2F and 2G) and the ventral part of the tail was gray or blackish (Figure 2G). The pattern on the head was the most variable, with white and black blotches. White color was mostly present on postoculars, preoculars, supralabials, sublabials, and the lower part of the rostral scale (Figures 2B, 3A, and 3C). The ventral part of the head was also white (Figure 2D). The eyes were always dark and the pupils were not visible. The individuals from Trebeshinë Mts. and Divjakë had the following numbers of head scales: one preocular, two postocular, one subocular, and eight supralabial head scales. Numbers of other body scales were as follows (Divjakë individual, male): 19 dorsal, 181 ventral, and 101 subcaudal scales. Caudal scales were divided. Total length of the body was 1130 mm, length of the tail 310 mm. Our described coloration was recorded in all age stages and in both sexes. All the individuals were found in typical Mediterranean habitats (Figure 2H) except the individual from Trebeshinë Mts., whose habitat consisted of mountain meadows at 1888 m a. s. l. This altitude represents probably the highest recorded elevation for this species.

According to our genetic analyses of the mitochondrial cyt *b* gene (1070 bp), the individual from Divjakë corresponded (100%) to haplotype HapH8 of *Hierophis gemonensis* (accession number in GenBank: JX315469; Mixoi, Axaias, Peloponnese, Greece; Kyriazi et al., 2013).

In the past, several species of Western Palearctic reptiles, especially snakes, have been regarded as taxonomically distinct based on their distinctive external morphology, e.g., different (black/melanistic) coloration (names are according to original description): *Testudo graeca terrestris* Forsskål, 1775; *Coluber prester* Linnaeus, 1761 i.e. melanistic aberration of *Vipera berus* (Linnaeus, 1758); *Vipera aspis atra* Meisner, 1820; *Natrix cetti* Gené, 1839; *Natrix natrix schweizeri* L. Müller, 1932; *Coluber gemonensis gyarosensis* Mertens, 1968, i.e. currently *Hierophis carbonarius* (Bonaparte 1833); *Coluber najadum kalymnensis* Schneider, 1979; *Vipera nikolskii* Vedmederja, Grubant & Rudayeva, 1986; or *Natrix megalocephala* Orlov & Tuniyev, 1987. Most of these names were later synonymized using molecular approaches that often opposed the previously recognized taxonomy (Utiger and Schätti, 2004; Kindler et al., 2013). On the other hand, there are snake species that showed little external morphological variation but deep genetic divergences, e.g., two species of whip snake that are melanistic in the adult stage, *Hierophis cypriensis* (Schätti, 1985) and *H. carbonarius* (Bonaparte, 1833), morphologically and ecologically similar to the closest relatives *H. gemonensis* and *H. viridiflavus* (Utiger and Schätti, 2004; Mezzasalma et al., 2015). *Hierophis gemonensis* was over time considered a monotypic species with one exception: melanistic snakes living on Gyaros Island, Greece, were classified as *H. gemonensis gyarosensis* (Dimitropoulos, 1986). However, they were later synonymized under *H. viridiflavus*/*carbonarius* based on genetic similarity that indicates its introduction on the island in ancient times (Utiger and Schätti, 2004). In light of the trend that started in the 18th century, when designation of almost every morphological variation was affected by taxonomy, it is surprising that such different individuals presented above had not been described as subspecies or varieties as were many other European

Table. Summary of characteristics of black-colored individuals recorded in the Balkans.

Number on the map	Date	Country	Locality	N	E	Altitude (m a.s.l.)	Sex	Age	Note	Photo number
1	1970	Croatia	Rožat	42.677°	18.128°	126	-	-	-	-
2	May 1979	Greece	Amfissia	38.481°	22.417°	137	-	-	-	-
3	1980	Greece	Agios Triada	39.718°	21.644°	503	-	-	-	-
4	1980	Greece	Damasi	39.709°	22.209°	133	-	-	-	-
5	1 May 2003	Greece	Tripoli	37.536°	22.366°	748	-	-	Two melanistic and 11 normally colored specimens	-
6	19 May 2007	Montenegro	Virpazar	42.238°	19.089°	4	-	Juvenile	Roadkill	Figure 3E
7	2013	Greece	Panteleimonas	40.005°	22.598°	103	-	-	-	-
8	28 July 2013	Albania	Trebeshinë Mts.	40.354°	20.094°	1888	-	Subadult	One melanistic and two other normally colored specimens	Figures 3C, 3D
9	21 April 2014	Greece	Platanovrisi	38.118°	21.763°	322	-	-	-	Figure 3H
10	4 April 2015	Greece	Vlasia	37.986°	21.899°	880	-	-	-	Figures 3F, 3G
11	21 May 2015	Montenegro	Danilovgrad	42.555°	19.110°	174	F	Adult	-	Figures 3A, 3B
12	25 September 2015	Albania	Divijakë, NP Karavastasë	40.989°	19.493°	1	M	Adult	Injury in the left lateral site	Figures 2A–2G

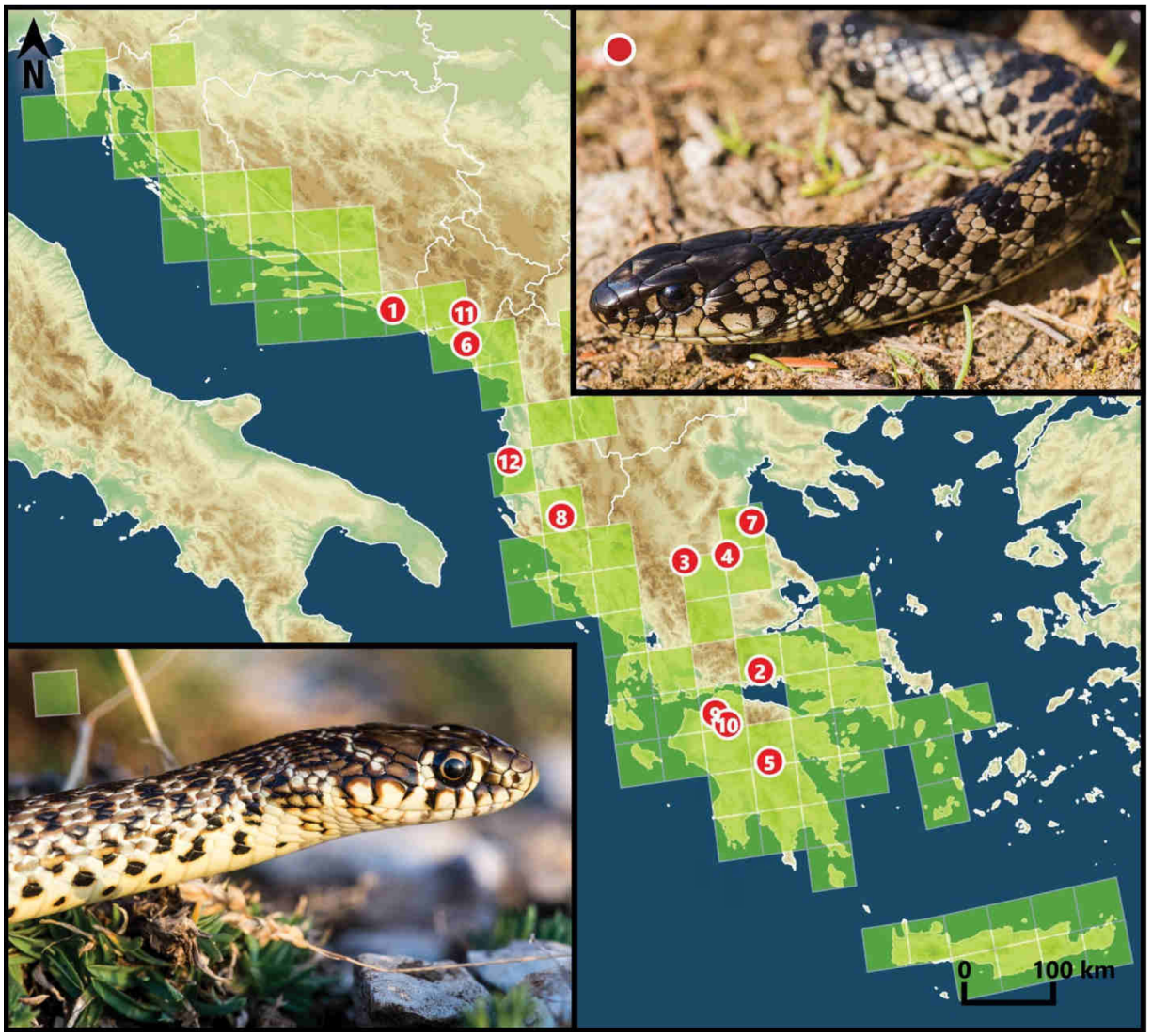

Figure 1. Records of black-colored specimens of *Hierophis gemonensis* in the southwestern Balkans summarized in the present study. The numbers on the map correspond to the Table. The distribution of the species in the Balkans is shown in green (according to Sillero et al., 2014). Insets show a typically colored snake (lower left, photo by E Mizsei) and a black-colored individual (top right, photo by D Jablonski), both photographed in Albania.

species (see Mertens and Wermuth, 1960). This can indicate that records of this dark color aberration in *H. gemonensis* are probably rare in nature and were overlooked in the past. However, when we compared morphological characters of the species presented by Henle (1993) with characters of the specimen from Divjakë-Karavasta NP, there were no major differences except the body coloration that is highly different from commonly colored *H. gemonensis* (compare insets in Figure 1).

As is known from the literature, related species of whip snakes of the genera *Dolichophis*, *Hierophis*, or *Platyceps* display certain levels of melanism. Apparently, the melanism or black coloration in European whip snakes is linked with the adult stage (*D. jugularis* - however, recently a black juvenile of *D. jugularis* was also recorded: Göçmen et al., 2015; *H. carbonarius*, *H. viridiflavus*: Zuffi, 2008) and it is taxonomically relevant, or it is randomly distributed in populations throughout the area of particular species probably due to specific current or past environmental processes (e.g., *D. caspius*, *Platyceps n. kalymnensis*: Foufopoulos et al., 1997; Cattaneo, 1998; Broggi, 2010). What is behind our described cases is only speculative without any experimental tests. In general, melanism or black coloration can be connected with crypsis, substrate coloration and humidity (increasing humidity causes higher pigment deposition), thermoregulation (e.g., secondary adaptation to colder climates during the Pleistocene), protection against

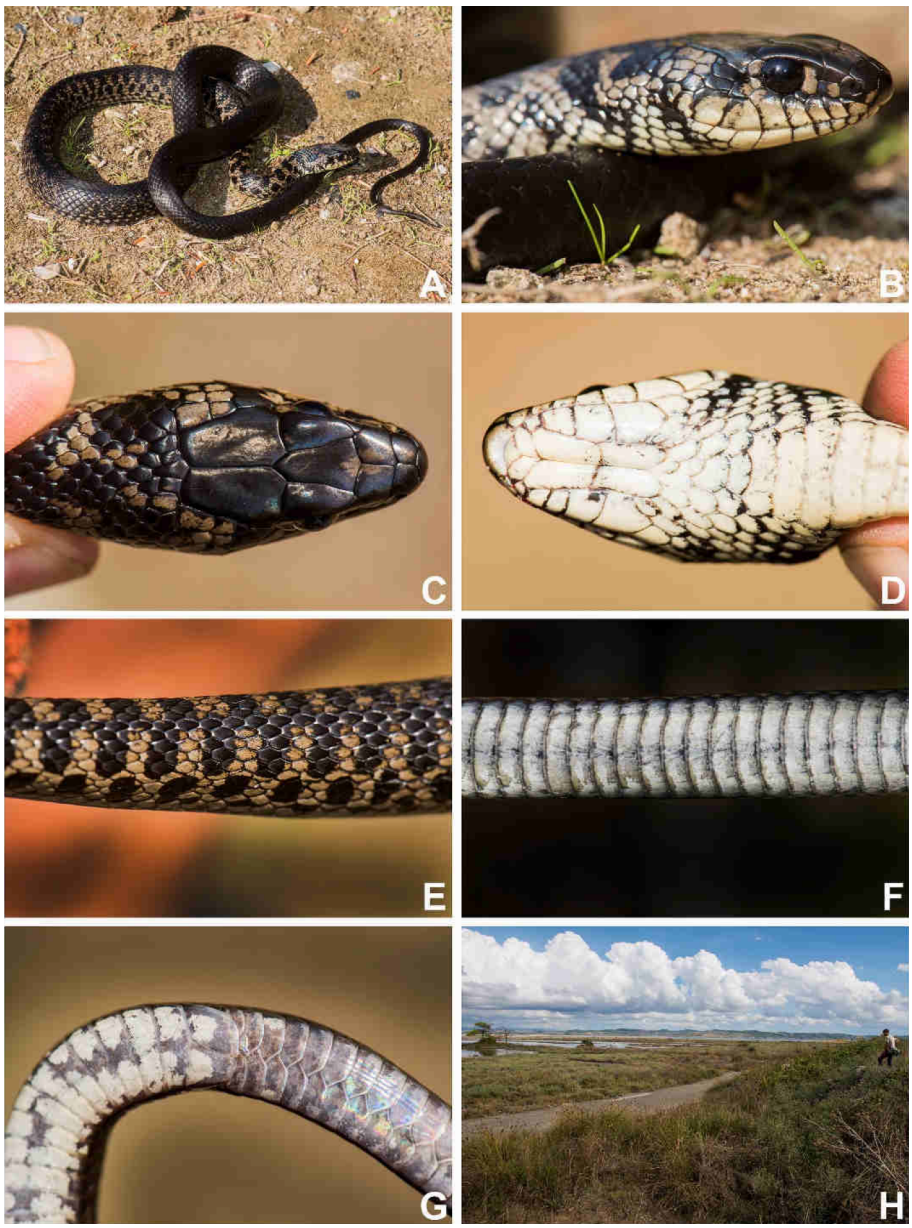

Figure 2. A black colored individual of *Hierophis gemonensis* from Divjakë-Karavasta NP, Albania. A – Overall view; B – detailed lateral view of the head; C – dorsal view of the head; D – ventrolateral view of the head; E – dorsal view of the first third of the body with zig-zag pattern; F – view of ventral scales in the middle part of the body; G – transition between the body and tail with anal and subcaudal scales; H – habitat of the specimen in Divjakë-Karavasta NP. All photos by D Jablonski.

solar radiation, or higher reproductive success, but it also brings some disadvantages such as high predation risk, or is even nonadaptive (Andrén and Nilson, 1981; Capula and Luiselli, 1994; Rosenblum, 2005; Millien et al., 2006; Lorioux et al., 2008). Luiselli (1995) discussed melanistic individuals of *Hierophis viridiflavus*/*carbonarius* especially regarding advantages for reproduction. This author also argued that the melanistic condition is present more frequently in females than males and that melanistic individuals attain larger body sizes than those that are normally colored. We have a small dataset to provide any relevant conclusions about the presence of this rare dark coloration in *H. gemonensis*. However, we could presume that both thermoregulation (a female from Trebeshinë Mts.) and reproductive/mating success (a big male from Divjakë) may play a role for melanism in whip snakes.

Figure 3. Black colored individuals of *Hierophis gemonensis* from the Balkans. A, B – Specimen from Danilovgrad, Montenegro (photo by A Simović); C, D – Trebeshinë Mts., Albania (E Mizsei); E - Virpazar, Montenegro (J Hill); F, G – Vlasia, Greece (E Tzoras); H – Platanovrisi, Greece (E Tzoras). For details see the Table.

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